PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/29, C07K 14/415, A61K 39/36 | A1

(11) International Publication Number: WO 95/27786

(43) International Publication Date: 19 October 1995 (19.10.95)

(21) International Application Number: PCT/US95/04249

(22) International Filing Date: 6 April 1995 (06.04.95)

(30) Priority Data:
08/226,248 8 April 1994 (08.04.94) US
08/350,225 6 December 1994 (06.12.94) US

(71) Applicant: IMMULOGIC PHARMACEUTICAL CORPORATION [US/US]; 610 Lincoln Street, Waltham, MA 02154 (US).

(72) Inventors: FRANZÉN, Henry, M.; 372 Charles River Road, Watertown, MA 02172 (US). POWERS, Stephen, Palmer; 2008 Stearns Hill Road, Waltham, MA 02154 (US). KUO, Mei-chang; 5 Cox Road, Winchester, MA 01890 (US). EVANS, Sean; 6 Lincoln Drive, Acton, MA 01720 (US). SHAKED, Ze'ev; 1038 Sierra Street, Berkeley, CA 94707 (US). CHEN, Xian; 755 Wellman Avenue, North Chelmsford, MA 01863 (US).

(74) Agents: VANSTONE, Darlene, A. et al.; Immulogic Pharmaceutical Corporation, 610 Lincoln Street, Waltham, MA 02154 (US).

(81) Designated States: AU, CA, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL FORMULATIONS FOR TREATING JAPANESE CEDAR POLLEN ALLERGY

PEPTIDE NAME:	AMINO ACID SEQUENCE
CJI-43.39	**DDAYSDDKSMKVTVAFNQFGDE**
CJr-24.5	**DKEPRARYGLVHVANNNYDPWTIEEE**
CJI-44.8	**DEEGAYFVSSGKYEGGNIYTKKEAFNVE**

(57) Abstract

The present invention provides novel peptides of *Cry j* I which have been modified as a part of a preformulation scheme to develop an optimized drug product for therapeutic treatment of humans suffering from allergy to Japanese cedar pollen allergen or a pollen allergen which is immunologically cross reactive Japanese cedar pollen allergen. Such modified peptides possess certain unique characteristics which render them particularly suitable for drug product formulation. The present invention further provides therapeutic compositions and multipeptide formulations which have been optimized to accommodate and maintain the unique characteristics of the modified *Cry j* I peptides and at the same time provide maximum therapeutic effect when used in therapeutic regimens for the treatment of Japanese cedar pollen allergy in humans.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/29, C07K 14/415, A61K 39/36 — A1

(11) International Publication Number: **WO 95/27786**

(43) International Publication Date: 19 October 1995 (19.10.95)

(21) International Application Number: PCT/US95/04249

(22) International Filing Date: 6 April 1995 (06.04.95)

(30) Priority Data:
08/226,248 8 April 1994 (08.04.94) US
08/350,225 6 December 1994 (06.12.94) US

(71) Applicant: IMMULOGIC PHARMACEUTICAL CORPORATION [US/US]; 610 Lincoln Street, Waltham, MA 02154 (US).

(72) Inventors: FRANZÉN, Henry, M.; 372 Charles River Road, Watertown, MA 02172 (US). POWERS, Stephen, Palmer; 2008 Stearns Hill Road, Waltham, MA 02154 (US). KUO, Mei-chang; 5 Cox Road, Winchester, MA 01890 (US). EVANS, Sean; 6 Lincoln Drive, Acton, MA 01720 (US). SHAKED, Ze'ev; 1038 Sierra Street, Berkeley, CA 94707 (US). CHEN, Xian; 755 Wellman Avenue, North Chelmsford, MA 01863 (US).

(74) Agents: VANSTONE, Darlene, A. et al.; Immulogic Pharmaceutical Corporation, 610 Lincoln Street, Waltham, MA 02154 (US).

(81) Designated States: AU, CA, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL FORMULATIONS FOR TREATING JAPANESE CEDAR POLLEN ALLERGY

PEPTIDE NAME:	AMINO ACID SEQUENCE
CJI-43.39	DDAYSDDKSMKVTVAFNQFGDE
CJr-24.5	DKEPRARYGLVHVANNNYDPWTIEEE
CJI-44.8	DEEGAYFVSSGKYEGGNIYTKKEAFNVE

(57) Abstract

The present invention provides novel peptides of *Cry j* I which have been modified as a part of a preformulation scheme to develop an optimized drug product for therapeutic treatment of humans suffering from allergy to Japanese cedar pollen allergen or a pollen allergen which is immunologically cross reactive Japanese cedar pollen allergen. Such modified peptides possess certain unique characteristics which render them particularly suitable for drug product formulation. The present invention further provides therapeutic compositions and multipeptide formulations which have been optimized to accommodate and maintain the unique characteristics of the modified *Cry j* I peptides and at the same time provide maximum therapeutic effect when used in therapeutic regimens for the treatment of Japanese cedar pollen allergy in humans.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

Pharmaceutical Formulations for Treating Japanese Cedar Pollen Allergy

Background of the Invention

Japanese cedar (Sugi; *Cryptomeria japonica*) pollinosis is one of the most important allergic diseases in Japan. The number of patients suffering from this disease is on the increase and in some areas, more than 10% of the population are affected.

The major allergen from Japanese cedar pollen has been purified and the amino acid sequence partially identified, and designated as Sugi basic protein (SBP) or *Cry j* I (Yasueda et al. (1983) *J. Allergy Clin. Immunol.* **71**: 77-86; and Taniai et al. (1988) *FEBS Letters* **239**: 329-332). *Cry j* I has been cloned and the full length nucleic acid sequence and the full length amino acid sequence of the *Cry j* I protein have been identified (WO 93/01213)

The *Cry j* I allergen found in *Cryptomeria japonica* has also been found to be cross-reactive with allergens in the pollen from other species of trees, including *Cupressus sempervirens*. Panzani et al. (*Annals of Allergy* **57**: 26-30 (1986)) reported that cross reactivity was detected between allergens in the pollens of *Cupressus sempervirens* and *Cryptomeria japonica* in skin testing, RAST and RAST inhibition. A 50 kDa allergen isolated from Mountain Cedar (*Juniperus sabinoides*, also known as *Juniperus ashei*) has an NH_2-terminal sequence (Gross et al., (1978) Scand. J. *Immunol.* **8**: 437-441) which is the same sequence as the first five amino acids of the NH-2 terminal end of the *Cry j* I allergen. The *Cry j* I allergen has also been found to be allergenically cross-reactive with the following species of trees: *Cupressus arizonica, Cupressus macrocarpa, Juniperus virginiana, Juniperus communis, Thuya orientalis,* and *Chamaecyparis obtusa.*

Treatment of Japanese cedar pollinosis by administration of Japanese cedar pollen extract to effect hyposensitization to the allergen has been attempted. Desensitization using Japanese cedar pollen extract, however, has drawbacks in that it can elicit anaphylaxis if high doses are used, whereas when low doses are used to avoid anaphylaxis, treatment must be continued for several years to build up a tolerance to the extract.

WO 94/01560 discloses improved compositions and methods for treatment of sensitivity to Japanese cedar pollen or to an immunologically cross reactive

pollen allergen which greatly minimizes the potential adverse side effects associated with desensitization therapy using Japanese cedar pollen extract. WO 94/01560 discloses isolated antigenic fragments or peptides derived from *Cry j* I which when administered to a Japanese cedar pollen-sensitive individual, or an individual allergic to an allergen cross-reactive with Japanese cedar pollen allergen, are capable of down regulating the allergic response of the individual to Japanese cedar pollen or such cross reactive allergen. Such down regulation of the allergic response of the individual results in diminution or alleviation of the classic symptoms of allergy including asthmatic symptoms induced by Japanese cedar pollen. Such antigenic fragments are disclosed as being capable of eliciting a T cell response such as stimulation (i.e. T cell proliferation or lymphokine secretion) and/or are capable of inducing T cell non-nonresponsiveness or reduced T cell responsiveness when challenged with Japanese cedar pollen allergen. In addition, WO 94/01560 discloses that the most preferred *Cry j* I peptides suitable for therapeutic use do not bind IgE specific for *Cry j* I, or bind IgE to a substantially lesser extent than the native *Cry j* I allergen, thereby reducing or eliminating the possibility of anaphylaxis in a treatment regimen which includes such peptides. Finally, WO 94/01560 discloses peptides which possess the characteristics described above.

As a result of extensive preformulation efforts, the present invention provides novel modified *Cry j* I peptides and novel combinations and formulations of modified *Cry j* I peptides which are optimal for preparation of a drug product suitable for use in treating Japanese cedar pollen allergy in humans and other mammals. Such *Cry j* I peptides and formulations thereof for use as an optimized human drug product have not previously been disclosed or contemplated.

Summary of the Invention

The present invention provides novel peptides of *Cry j* I which have been modified as a part of a preformulation scheme to develop an optimized drug product for therapeutic treatment of humans suffering from allergy to Japanese cedar pollen allergen or a pollen allergen which is immunologically cross reactive Japanese cedar pollen allergen. Such modified peptides possess certain unique characteristics which render them particularly suitable for drug product formulation. The present invention further provides therapeutic compositions and multipeptide formulations which have been optimized to accommodate and maintain the unique characteristics of the modified *Cry j* I peptides and at the

same time provide maximum therapeutic effect when used in therapeutic regimens for the treatment of Japanese cedar pollen allergy in humans.

Description of the Drawings

Fig. 1 shows the complete cDNA sequence for *Cry j* I (SEQ. ID. NO: 39) which is composed of 1312 nucleotides, including 66 nucleotides of 5' untranslated sequence, an open reading frame starting with the codon for an initiating methionine of 1122 nucleotides, and a 3' untranslated region. Fig. 1 also shows the deduced amino acid sequence of *Cry j* I (SEQ. ID. NO: 40);

Fig. 2 shows 35 overlapping 20mer peptides which overlap by 10 derived from *Cry j* I.

Fig. 3 shows the amino acid sequences of the novel "unique" peptides of the invention.

Fig. 4 is a graphic representation depicting responses of T cell lines from twenty-five patients primed in vitro with purified native *Cry j* I and analyzed for response to the 35 overlapping 20 mer *Cry j* I peptides shown in Fig. 2, by percent of responses (positive) with an S.I of at least two (shown over each bar), the mean stimulation index of positive response for the peptide (shown over each bar in parenthesis) and the positivity index (Y axis);

Fig. 5 is a graphic representation depicting T cell responses to the overlapping *Cry j* I peptides shown in Fig. 2, and the novel "unique" peptides of the invention shown in Fig. 3. The mean S.I. shown above each bar (in parenthesis) as well as the percentage of responses, the positivity index (mean S.I. multiplied by percentage of responses) is the Y axis.

Fig. 6a is a pH solubility profile of CJI-24.5 (SEQ. ID. NO: 36) (desalted) in 50mM phosphate buffer with 5% mannitol and 22°C, with 95.2% estimated peptide content and 2.7% acetate.

Fig. 6b is a pH solubility profile of CJI-43.39 (SEQ. ID. NO: 37) in 50mM phosphate buffer with 5% mannitol and 22°C, with 83.5% estimated peptide content and 1.9% acetate.

Fig. 6c is a pH solubility profile of CJI-44.8 (SEQ. ID. NO: 38)in 50mM phosphate buffer with 5% mannitol and 22°C, with 86.% estimated peptide content and 1.5% acetate.

Detailed Description of the Invention

The present invention provides novel peptides of *Cry j* I which have been modified as a part of a preformulation scheme to develop an optimized drug product for therapeutic treatment of humans suffering from allergy to Japanese cedar pollen allergen or an allergen which is immunologically cross reactive with Japanese cedar pollen allergen (e.g. *Cupressus sempervirens, Juniperus sabinoides* (also known as *Juniperus ashei) Cupressus arizonica, Cupressus macrocarpa, Juniperus virginiana, Juniperus communis, Thuya orientalis,* and *Chamaecyparis obtusa).* Such modified peptides possess certain unique characteristics which render them particularly suitable for drug product formulation, and may be referred to herein as "unique" peptides.

In accordance with pharmaceutical chemistry, preformulation is the process of optimizing a drug through determination and/or definition of those physical and chemical properties considered important in the formulation of a stable, effective, and safe dosage form. The possible interactions with the various components intended for use in the final drug product are also considered. Preformulation is an intensive effort that includes the study of such parameters as solubility, pH profile of stability, and drug-excipient interactions, which may have a profound effect on a drug's physiological availability and physical and chemical stability. The data obtained from such studies are integrated with those obtained from preliminary pharmacological and biochemical studies of the active drug component thus providing information that permits the selection of the best drug form, and the most desirable excipients for use in its development.

The development of an optimum formulation of active drug component and excipients is complex and many factors influence formulation properties. The high degree of uniformity, the physiological availability and the therapeutic quality expected of pharmaceuticals can only be achieved by considerable effort and expertise. Flexibility is also an important factor in preformulation. Numerous excipients, stabilizers counter ions and the like may have to be tested in order to find those compatible with the active drug component of the formulation. Multiple modifications of the active component may become necessary to

successfully formulate a drug product. Such modifications must not effect the overall therapeutic effectiveness of the drug but at the same time, must render the drug more suitable for formulation.

As a part of a preformulation scheme to provide an optimized drug product suitable for use in humans and other mammals for treating sensitivity to *Cry j* I, it was determined that the active component (a peptide or candidate peptide) in such formulation should possess the following characteristics which would render such peptides "unique" among all of the possible peptides derived from the *Cry j* I sequence. First, a unique peptide should alone or in combination with other unique peptides comprise a sufficient percentage of the T cell reactivity of the *Cry j* I protein allergen to induce T cell nonresponsiveness or reduced T cell responsiveness in a substantial percentage of the individuals sensitive to *Cry j* I protein allergen. Second, the candidate peptide should possess the characteristic of "superior solubility" which is defined herein as solubility of greater than 5 mg/ml at a pH in the pH range of pH 6 to pH 8 in an aqueous buffer. Third, the peptide is stable in an aqueous buffer at a pH in the pH range of pH 6 to pH 8. Candidate peptides which have been determined to be "unique" peptides of the invention are CJI-24.5 (SEQ. ID. NO: 37), CJI-43.39 (SEQ. ID. NO: 36), and CJI-44.8 (SEQ. ID. NO: 38), all as shown in Fig. 3.

In accordance with the first characteristic, those peptides found to elicit a T cell response such as T cell proliferation or lymphokine secretion (i.e. comprise at least one T cell epitope), or induce T cell non-responsiveness or reduced T cell responsiveness are understood to have T cell reactivity. T cell epitopes are believed to be involved in initiation and perpetuation of the immune response to a protein allergen which is responsible for the clinical symptoms of allergy. These T cell epitopes are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of antibodies. One isotype of these antibodies, IgE, is fundamentally important to the development of allergic symptoms and its production is influenced early in the cascade of events, at the level of the T helper cell, by the nature of the lymphokines secreted. A T cell epitope is the basic element or smallest unit of recognition by a T cell receptor, where the epitope comprises amino acids essential to receptor recognition. It is believed that

exposure of Japanese cedar pollen patients to isolated *Cry j* I peptides which comprise at least one T cell epitope may cause T cell non-responsiveness of appropriate T cell subpopulations such that they become unresponsive or have reduced responsiveness to the protein allergen and do not participate in stimulating an immune response upon such exposure for example, via anergy, tolerance, or apoptosis, the ability to modify the lymphokine secretion profile as compared with exposure to the naturally occurring autoantigen; and /or the ability to cause induction of T suppresser cells.

To determine peptides having T cell reactivity and comprising at least one T cell epitope, isolated peptides are tested by, for example, T cell biology techniques, to determine whether the peptides elicit a T cell response or induce T cell non-responsiveness. As discussed in the Examples human T cell stimulating activity can be tested by culturing T cells obtained from an individual sensitive to Japanese cedar pollen allergen, (i.e., an individual who has an IgE mediated immune response to Japanese cedar pollen allergen) with a peptide or modified peptide derived from *Cry j* I and determining whether proliferation of T cells occurs in response to the peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for responses by T cells to peptides can be calculated as the maximum counts per minute (CPM) in response to a peptide divided by the control CPM. A stimulation index (S.I.) equal to or greater than two times the background level is considered "positive". Positive results are used to calculate the mean stimulation index for each peptide for the group of patients tested. Peptides suitable as candidates for formulation into a final drug product have a mean T cell stimulation index of greater than or equal to 2.0 and preferably higher, (e.g. at least 2.5, more preferably at least 3.5, more preferably at least 4.0, more preferably at least 5, even more preferably at least 7 and most preferably at least about 9).

For therapeutic purposes, candidate peptides are recognized by at least 10%, more preferably at least 20%, more preferably at least 30% and even more preferably at least 40% or more of individuals in a population of individuals sensitive to Japanese cedar pollen. In addition, preferred candidate peptides have a positivity index (P.I.) of at least about 100, more preferably at least about 250 and most preferably at least about 350. The positivity index for a peptide is determined by multiplying the mean T cell stimulation index by the percent of individuals, in a population of individuals sensitive to Japanese cedar pollen (e.g., preferably at least 15 individuals, more preferably at least 30 individuals or more),

who have a T cell stimulation index to such peptide of at least 2.0. Thus, the positivity index represents both the strength of a T cell response to a peptide (S.I.) and the frequency of a T cell response to a peptide in a population of individuals sensitive to Japanese cedar pollen.

To determine whether a peptide (candidate peptide) or a combination of candidate peptides are likely to comprise a sufficient percentage of the T cell reactivity of the *Cry j* I to induce T cell nonresponsiveness in a substantial percentage of a population of individuals sensitive to *Cry j* I, an algorithm can be used. In accordance with one such algorithm, a set of overlapping peptides is produced by systematically dividing *Cry j* I into at least two overlapping peptide regions of desired lengths (e.g., of about 12-30 amino acid residues in length, preferably not longer than about 25 amino acid residues in length with about 5-15 amino acid residues of overlap). For example, see Fig. 2. This division into peptide regions can be arbitrary, can be made according to an algorithm, or can be wholly or partially based on regions of *Cry j* I known to comprise at least one T cell epitope. Preferably, at least 50% of the entire *Cry j* I protein sequence and more preferably, the entire protein sequence of *Cry j* I is divided into two or more peptides. A human T cell stimulation index is determined for each of the peptides in an *in vitro* T cell proliferation assay as described herein for each individual tested in a population of individuals sensitive to the protein antigen. For example, see Fig. 4. A candidate peptide or combination of candidate peptides is selected based, at least in part, on the mean human T cell stimulation index of the candidate peptide in the set of peptides tested and the positivity index of the candidate peptide in the set of peptides tested. For example see, Fig. 5. The human T cell stimulation index for the candidate peptide(s) is summed. For each individual, the human T cell stimulation index for the candidate peptide(s) is divided by the sum of the human T cells stimulation indices of the remaining peptides in the set of peptides tested to determine a percent of T cell reactivity as shown below:

$$(1)\ \%\ \text{T Cell Reactivity of a candidate peptide(s)} = \frac{\text{Candidate S.I.}}{\text{Sum of S.I. of the set of Overlapping peptides}} \times 100$$

Alternatively, the presence of T cell epitopes in the candidate peptide dependent on amino acids residues in an overlapping peptide located at either the

N-terminus or C-terminus of the candidate peptide in the amino acid sequence of the protein antigen, but which epitopes are not present in the candidate peptide can be considered in calculating the percent of T cell reactivity in the candidate peptide by use of the following formula:

(2) % T Cell Reactivity of a candidate peptide(s) =

$$\frac{N_T \text{ flanking peptide S.I.} + \text{Candidate peptide S.I.} + C_T \text{ flanking peptide S.I.}}{\text{Sum of S.I. of the set of overlapping peptides}} \times 100$$

In this formula, "N_T flanking peptide" refers to a peptide which comprises amino acid residues which overlap with amino acid residues located at the N-terminus of the candidate peptide in the amino acid sequence of the protein antigen from which the peptide is derived; "C_T flanking peptide" refers to a peptide which comprises amino acid residues which overlap with amino acid residues located a the C-terminus of the candidate peptide in the amino acid sequence of the protein antigen from which the peptide is derived. In this calculation stimulation indices for the candidate peptide, the N-terminal flanking peptide and the C-terminal flanking peptide are added and divided by the sum total of the stimulation indices for the entire set of overlapping peptides obtain a percent of T cell reactivity for the candidate peptide. If a combination of two or more candidate peptides is selected each of which contains amino acid residues which overlap, this calculation cannot be used to determine a percent of T cell reactivity for each candidate peptide separately. However, a total percent of T cell reactivity for the combination of candidate peptides can be obtained. In this situation, the stimulation indices for all of the candidate peptides which overlap is included in the calculation.

The values obtained for the percentage of T cell reactivity for the candidate peptide or combination of peptides in each individual tested can be expressed as a range of the lower and higher values of the results of the above described calculations. By either of the above calculations, the percent is obtained for at least about twenty (20) and preferably at least about thirty (30) individuals sensitive to the protein antigen and a mean percent is determined. For use in the compositions of the invention, the candidate peptide or combination of candidate peptides has the following criteria: (1) the candidate peptide or combination of

candidate peptides has a mean percent of at least about 10%, preferably at least about 20%, more preferably at least about 30%, more preferably at least about 40% and more preferably at least about 50-60% or greater; and (2) in the population of individuals tested at least about 60%, preferably at least about 75%, and more preferably at least about 90-100% have positive T call responses (S.I. equal to or greater than 2.0) in response to the candidate peptide or combination of candidate peptides. A candidate peptide or combination of candidate peptides meeting the above criteria is likely to comprise a sufficient percentage of the T cell reactivity to *Cry j* I to induce T cell non-responsiveness or reduced T cell responsiveness in a substantial percentage of a population of individuals sensitive to *Cry j* I.

As an illustrative embodiment of the above-described algorithm, a set of overlapping peptides and candidate peptides, CJI-24.5, CJI-23.39 and CJI-44.8, derived from *Cry j* I were produced and tested. Secondary T cell cultures determined to be reactive with *Cry j* I protein antigen were derived from 36 *Cry j* I-allergic subjects and analyzed for reactivity to the overlapping set of peptides in an in vitro T cell proliferation assay as described herein. The results are shown in Fig. 5. The highest stimulation index greater than or equal to 2.0 in response to each peptide was recorded for each subject tested. The data were then analyzed by the equations above. The results and calculations of the percent of T cell reactivity for a single *Cry j* I-allergic subject are shown below using formulas (1) and (2).

T CELL REACTIVITY FOR PATIENT 1308

PEPTIDE STIMULATION INDEX

CJ1-1 (SEQ. ID. NO: 1)	10.9
CJ1-2 (SEQ. ID. NO: 2)	16.1
CJ1-3 (SEQ. ID. NO: 3)	8.8
CJ1-4 (SEQ. ID. NO: 4)	0
CJ1-5 (SEQ. ID. NO: 5)	3.2
CJ1-6 (SEQ. ID. NO: 6)	0
CJ1-7 (SEQ. ID. NO: 7)	2.5
CJ1-8 (SEQ. ID. NO: 8)	0
CJ1-41 (SEQ. ID. NO: 41)	8.9
CJ1-11 (SEQ. ID. NO: 11)	0
CJ1-12 (SEQ. ID. NO: 12)	0
CJ1-13 (SEQ. ID. NO: 13)	0
CJ1-14 (SEQ. ID. NO: 14)	0
CJ1-15 (SEQ. ID. NO: 15)	0
CJ1-42.5 (SEQ. ID. NO: 42)	17.6
CJ1-18 (SEQ. ID. NO: 18)	0
CJ1-19 (SEQ. ID. NO: 19)	0
CJ1-20 (SEQ. ID. NO: 20)	0
CJ1-21 (SEQ. ID. NO: 21)	0
CJ1-43.39 (SEQ. ID. NO: 36)	25.6
CJ1-23 (SEQ. ID. NO: 23)	5.3
CJ1-24.5 (SEQ. ID. NO: 37)	6.9
CJ1-25 (SEQ. ID. NO: 25)	9.4
CJ1-26 (SEQ. ID. NO: 26)	11.9
CJ1-27 (SEQ. ID. NO: 27)	5.5
CJ1-28 (SEQ. ID. NO: 28)	0
CJ1-29 (SEQ. ID. NO: 39)	2.9
CJ1-30 (SEQ. ID. NO: 30)	0
CJ1-44.8 (SEQ. ID. NO: 38)	21.5
CJ1-33 (SEQ. ID. NO: 33)	20.9
CJ1-34 (SEQ. ID. NO: 34)	17.8
CJ1-35 (SEQ. ID. NO: 35)	0

SUM OF STIMULATION INDICES: 195.7 (DENOMINATOR)

% Reactivity of Peptide 44.8 for patient 1308

(1) $$\frac{\text{CJ1 - 44.8 (S.I.)}}{195.7} = \frac{21.5}{195.7} \times 100 = 11\%$$

(2) $$\frac{\text{CJ1 - 30 + CJ1-44.8 + CJ1-33 (S.I.s)}}{195.7} = \frac{0 + 21.5 + 20.9}{195.7} \times 100 = 21.7\%$$

Therefore the estimated range of T cell reactivity for Peptide 44.8 (SEQ. ID. NO: 38) for this patient is 11%-21.7% of the total reactivity of the Cry j I protein. The above calculation is repeated for any potential candidate peptides. In the population of 36 Cry j I-allergic subjects tested the following results were obtained:

Candidate Peptides	Range of mean percentage T Cell Reactivity	Frequency of response at least one peptide
CJ1-24.5 + CJ1-43.3 + CJ1-44.8	36-53%	97%

Thus, the combination of the three candidate peptides of the invention are well within the desired range for possessing, in combination, sufficient T cell reactivity of *Cry j* I, and therefore, meet the first characteristic of a "unique" peptide of the invention.

For the treatment of allergy in accordance with the methods of the invention, it is preferred that a peptide used in conjunction therewith does not bind immunoglobulin E (IgE) or binds IgE to a substantially lesser extent (i.e. at least 100-fold less binding and more preferably, at least 1,000-fold less binding) than the *Cry j* I protein allergen from which the peptide is derived binds IgE. The major complications of standard immunotherapy are IgE-mediated responses such as anaphylaxis. Immunoglobulin E is a mediator of anaphylactic reactions which result from the binding and cross-linking of antigen to IgE on mast cells or basophils and the release of mediators (e.g., histamine, serotonin, eosinophil chemotacic factors) in allergic ("atopic") patients. Thus, anaphylaxis in a substantial percentage of a population of individuals sensitive to the allergen

being treated could be avoided by the use in immunotherapy of a peptide or peptides which do not bind IgE in a substantial percentage (e.g., at least about 75%) of a population of individuals sensitive to *Cry j* I, or if the peptide binds IgE, such binding does not result in the release of mediators from mast cells or basophils. The risk of anaphylaxis could be reduced by the use in immunotherapy of a peptide or peptides which have reduced IgE binding. IgE binding may be tested, for example by direct ELISA or capture ELISA. Moreover, peptides which have minimal IgE stimulating activity are desirable for therapeutic effectiveness. Minimal IgE stimulating activity refers to IgE production that is less than the amount of IgE production and/or IL-4 production stimulated by the native *Cry j* I protein allergen. If a peptide binds IgE, it is preferable that such binding does not result in the release of mediators (e.g. histamines) from mast cells or basophils. To determine whether a peptide that binds IgE causes the release of mediators, a histamine release assay can be performed using standard reagents and protocols obtained for example, from Amac, Inc. (Westbrook, ME). Briefly, a buffered solution of a peptide to be tested is combined with an equal volume of whole heparinized blood from an allergic subject. After mixing and incubation, the cells are pelleted and the supernatants are processed and analyzed using a radio immunoassay to determine the amount of histamine released. As described in Example 3, candidate peptides of the invention, 24.5 (SEQ. ID. NO: 37), 43.39 (SEQ. ID. NO: 36), and 44.8 (SEQ. ID. NO: 38), do not appear to bind IgE .

The second characteristic for a unique peptide is that of "superior solubility" which was defined earlier as being solubility of greater than 5 mg/ml at a pH in the pH range of pH 6 to pH 8. Solubility at a pH in a physiologically acceptable pH range (e.g. pH 6 to pH 8) is particularly important when formulating a multipeptide therapeutic for injection. Administration of a soluble drug product in a physiologically acceptable pH range by intravenous or subcutaneous injection provides 100% bioavailability of the drug component to the physiological system into which the drug is being introduced. Thus, it is necessary that a drug product intended for injection be fluid to the extent that easy syringability exists, and the active component be soluble as well if maximum therapeutic effect is to be achieved. Solubility is also useful when formulating compositions to be administered via other modes of administration such as by oral administration (tablet, aerosol, sublingual), or sustained release preparations and formulations.

Proteins and peptides may be difficult to formulate into soluble compositions as a peptide may not be soluble in any desirable pH range or may be soluble in only a narrow pH range. It is particularly difficult when multiple peptides are being formulated together into a single multipeptide formulation, as each peptide may be soluble in a pH range which does not overlap with those of the other peptides in the formulation. As a result, it is the requirement of "superior solubility" which requires the most formulation flexibility in that considerable modification of the targeted candidate peptides may be necessary to successfully formulate a multipeptide drug product.

The unique peptides of the invention are the product of multiple amino acid modifications of the original targeted candidate peptide sequence ("parent") from which the modified unique peptides of the invention were originally derived for the purpose of finding a peptide which fits the characteristic of "superior solubility". For example, the amino acid sequence of CJI-44.8 (SEQ. ID. NO: 38) was derived from the protein sequence of *Cry j* I (SEQ. ID. NO: 40) by first identifying those regions of the parent protein (Fig. 1) with high T cell reactivity using the set of overlapping peptides 20-mers as discussed in Example 1, and shown in Fig. 2, which covered the entire sequence. Two of these peptides, CJI-31 (SEQ. ID. NO: 31) and CJI-32 (SEQ. ID. NO: 32), individually exhibited high T-cell reactivity. Since these peptides were adjacent to each other in the native protein sequence (Fig. 1) and overlapped by 10 residues, a peptide, CJI-44, was synthesized to capture the total T cell reactivity of both peptides. CJI-44 is a peptide 30-mer having the amino acid sequence DEEGAYFVSSGKYEGGNIYTKKEAEFNVE (SEQ. ID. NO: 43) which contains all of the sequence present in the two 20-mers CJI-31 (SEQ. ID. NO: 31) and CJI-32 (SEQ. ID. NO: 32). However, although CJI-44 (SEQ. ID. NO: 43) possessed T cell reactivity of the two 20-mers, CJI-31 (SEQ. ID. NO: 31) and CJI-32 (SEQ. ID. NO: 32), when the solubility of CJI-44 (SEQ. ID. NO: 43) was tested it had a solubility much lower than the 5 mg/ml solubility required for a "unique" peptide of the invention.

Thus, further attempts were made to increase solubility by truncation at the N-terminus portion of CJI-44 which resulted in CJI-44.1 (NGAYFVSSGKYEGGNIYTKKEAFNVE) (SEQ. ID. NO: 44). Additional truncation of two C-terminal residues yielded 44.2 (NGAYFVSSGKYEGGNIYTKKEAFN) (SEQ. ID. NO: 45). However, although solubility was improved in these sequences it still did not reach the standard of

"superior solubility". Thus, 44.2 (SEQ. ID. NO: 45) was further modified by the addition of charged (hydrophilic residues) to the N-terminus and by replacement of the hydrophobic residue Val with the less hydrophobic residue Ala. Two of the resulting analogs, CJI-44.5 (DENGAYFVSSGKYEGGNIYTKKEAFNAE) (SEQ. ID. NO: 46) and CJI-44.6 (DEENGAYFVSSGKYEGGNIYTKKEAFNVE) (SEQ. ID. NO: 47), showed increased solubility to using a "single pH point protocol procedure" (e.g. a protocol procedure wherein determinations of solubility were made at a single pH in 100 mM sodium phosphate buffer without mannitol under constant agitation) Two additional analogs were constructed in which the residue Asn was deleted. Of these two analogs, CJI-44.7 (DEGAYFVSSGKYEGGNIYTKKEAFNAE) (SEQ. ID. NO: 48) and CJI-44.8 (DEEGAYFVSSGKYEGGNIYTKKEAFNVE) (SEQ. ID. NO: 38), CJI-44.8 was very soluble in the "single pH point protocol" and achieved "superior solubility" in the "pH range protocol procedure" (i.e. wherein solubility is measured as a function of pH in 50 mM sodium phosphate containing 5% mannitol with no agitation after initial mixing). CJ1-44.8 (SEQ. ID. NO: 38) was stable and soluble at greater than 5 mg/ml over the pH range pH6-pH8 in an aqueous buffer (see, Example 4 and Fig. 6c).

Peptide CJI-44.8 (SEQ. ID. NO: 38) was determined to be a "unique" peptide after confirmation that it also retained a T-cell reactivity similar to its parent peptides, CJI-31 (SEQ. ID. NO: 31), CJI-32 (SEQ. ID. NO: 32)and CJI-44 (SEQ. ID. NO: 43) (see Example 2), and confirmation of its stability as is discussed below. Development of the other "unique" peptides, CJI-24.5 (SEQ. ID. NO: 37) and CJI-43.39 (SEQ. ID. NO: 36), followed a process similar to that described above for CJI-44.8 (SEQ. ID. NO: 38).

The third criteria which the unique peptides of this invention must meet is stability at a physiologically acceptable pH in the range of pH 6 to pH 8. It must be stable under the conditions of manufacture and storage, and under conditions of reconstitution if necessary. Stability testing establishes the time period for which the integrity, quality and purity of the drug product is preserved in its finished dosage form. Stability testing may be performed concurrently with solubility studies as discussed in Example 4. Each of the candidate peptides of the invention remained stable (e.g. no significant degradation) at a physiologically pH in a pH range from pH 6-pH 8, at room temperature for at least 24 hours.

Therefore, candidate peptides CJI-24.5 (SEQ. ID. NO: 37), CJI-43.39 (SEQ. ID. NO: 36), and CJI-44.8 (SEQ. ID. NO: 38) possess each of the three

required "unique" characteristics outlined above, indicating that this combination of peptides is suitable for formulation as an optimized therapeutic drug product for administration to humans for treatment of allergy to Japanese cedar pollen allergen.

Highly purified peptides of this invention, may be produced synthetically by chemical synthesis using standard techniques. Various methods of chemically synthesizing peptides are known in the art such as solid phase synthesis which has been fully or semi automated on commercially available peptide synthesizers. Synthetically produced peptides may then be purified to homogeneity (i.e. at least 90%, more preferably at least 95% and even more preferably at least 97% purity), free from all other polypeptides and contaminants using any number of techniques known in the literature for protein purification.

In accordance with one procedure for producing highly purified homogenous peptides of the invention, a peptide produced by synthetic chemical means (either anchored to a polymer support "solid phase synthesis" or by conventional homogenous chemical reactions "solution synthesis") may be purified by preparative reverse phase chromatography. In this method, the synthetically produced peptide in "crude" form is dissolved in an appropriate solvent (typically an aqueous buffer) and applied to a separation column (typically a reverse phase silica based media, in addition, polymer or carbon based media may be used). Peptide is eluted from the column by increasing the concentration of an organic component (typically acetonitrile or methanol) in an aqueous buffer (typically TFA, triethylamine phosphate, acetate or similar buffer). Fractions of the eluate will be collected and analyzed by appropriate analytical methods (typically reverse phase HPLC or CZE chromatography). Those fractions having the required homogeneity will be pooled. The counter ion present may be changed by additional reverse phase chromatography in the salt of choice or by ion exchange resins. The peptide may then be isolated as its acetate or other appropriate salt. The peptide is then filtered and the water removed (typically by lyophilization) to give a homogenous peptide composition containing at least 90%, more preferably at least 95% and even more preferably at least 97% of the required peptide component. Optionally, or in conjunction with reverse phase HPLC as described above, purification may be accomplished by affinity chromatography, ion exchange, size exclusion, counter current or normal phase separation systems, or any combination of these methods. Peptide may

additionally be concentrated using ultra filtration, rotary evaporation, precipitation, dialysis or other similar techniques.

The highly purified homogenous peptide composition is then characterized by any of the following techniques or combinations thereof: a) mass spectroscopy to determine molecular weight to check peptide identity; b) amino acid analysis to check the identity of the peptide via amino acid composition; c) amino acid sequencing (using an automated protein sequencer or manually) to confirm the defined sequence of amino acid residues; d) HPLC (multiple systems if desired) used to check peptide identity and purity (i.e. identifies peptide impurities); e) water content to determine the water concentration of the peptide compositions; f) ion content to determine the presence of salts in the peptide composition; and g) residual organics to check for the presence of residual organic reagents, starting materials, and/or organic contaminants.

A peptide of the invention may also be produced by recombinant DNA techniques in a host cell transformed with a nucleic acid sequence coding for such peptide. When produced by recombinant techniques, host cells transformed with nucleic acid encoding the desired peptide are cultured in a medium suitable for the cells and isolated peptides can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying peptides and proteins including ion-exchange chromatography, ultra filtration, electrophoresis or immunopurification with antibodies specific for the desired peptide. Peptides produced recombinantly may be isolated and purified to homogeneity, free of cellular material, other polypeptides or culture medium for use in accordance with the methods described above for synthetically produced peptides.

In certain limited circumstances, peptides of this invention may also be produced by chemical or enzymatic cleavage of a highly purified full length or native protein of which the sites of chemical digest or enzymatic cleavage have been predetermined and the resulting digest is reproducible. Peptides having defined amino acid sequences can be highly purified and isolated free of any other poly peptides or contaminants present in the enzymatic or chemical digest by any of the procedures described above for highly purified, and isolated synthetically or recombinantly produced peptides.

The present invention also pertains to therapeutic compositions and multipeptide therapeutic formulations comprising the unique peptides of the invention. Therapeutic compositions of the invention may comprise one or more of the unique peptides of the invention which may be administered simultaneously

or sequentially as single treatment episode for treatment of allergy to Japanese cedar pollen allergen in a human or other mammal. Such a treatment regimen may not necessarily be a physical mixture of more than one peptide of the invention, but does comprise a combination of such peptides administered simultaneously or sequentially as a single treatment episode in order to achieve the maximum therapeutic effect the combination of the unique peptides, CJI-24.5 (SEQ. ID. NO: 37), CJI-43.39 (SEQ. ID. NO: 36), and CJI-44.8 (SEQ. ID. NO: 38), provide (e.g. solubility and stability at a pH in an acceptable physiological pH range (preferably pH 7.0 to pH 7.5) as well as covering 36-53% T cell reactivity in 97% of the patients tested).

Therapeutic compositions of the invention comprise one or more of peptides CJI-24.5 (SEQ. ID. NO: 37), CJI-43.39 (SEQ. ID. NO: 36), and CJI-44.8 (SEQ. ID. NO: 38) and also comprise one or more pharmaceutically acceptable carriers such as excipients which are compatible with peptide or peptides present in a single composition. When the composition is a multipeptide formulation, the pharmaceutically acceptable carrier must be compatible with all of the peptides in the multipeptide formulation. Preferred excipients include but are not limited to sterile water, sodium phosphate, mannitol, or both sodium phosphate and mannitol or any combination thereof. Other suitable excipients include but are not limited to sorbitol, sucrose, dextrose, lactose dextran and PVP. In addition, pharmaceutically acceptable counter ions may be added during the preparation of the multipeptide formulation. Examples of pharmaceutically acceptable counter ions include acetate, HCl, and citrate.

A therapeutic composition of the invention should be sterile, stable under conditions of manufacture, storage, distribution and use and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. A preferred means for manufacturing a therapeutic compositions of the invention in order to maintain the integrity of the composition (i.e. prevent contamination, prolong storage, etc.) is to prepare the formulation of peptide and pharmaceutically acceptable carrier(s) such that the composition may be in the form of a lyophilized powder which is reconstituted in a pharmaceutically acceptable carrier, such as sterile water, just prior to use. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying, freeze-drying or spin drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

A preferred multipeptide formulation comprises unique *Cry j* I peptides CJ1-24.5 (SEQ. ID. NO: 37), CJ1-43.39 (SEQ. ID. NO: 36) and CJ1-44.8 (SEQ. ID. NO: 38) and sodium phosphate and mannitol. For this embodiment, a suitable counter ion such as acetate may be added during the preparation of the formulation, and the formulation is preferably prepared in the form of a lyophilized powder which is reconstituted in a physiologically acceptable carrier, such as sterile water, prior to use. One, non-limiting example of a preferred multipeptide formulation of the invention is described below. The *Cry j* I peptides CJ1-24.5 (SEQ. ID. NO: 37), CJ1-43.39 (SEQ. ID. NO: 36) and CJ1-44.8 (SEQ. ID. NO: 38) will preferably be combined during manufacturing with the appropriate counter ion to produce a vial containing a sterile, pyrogen free, lyophilized powder having the following composition:

Active:	*Cry j* I peptides CJ1-24.5 (SEQ. ID. NO: 37), CJ1-43.39 (SEQ. ID. NO: 36) and CJ1-44.8 (SEQ. ID. NO: 38) In concentration of 0.75mg per peptide
Inactives:	0.05 M Sodium Phosphate pH 6.0-8.0 5% w/v Mannitol, U.S.P.
Diluent:	Sterile Water for Injection, U.S.P. (initial reconstitution) 0.9% Sodium Chloride for Injection (dilution beyond initial reconstitution)
Final pH	pH 7.0-pH 7.5

The multipeptide formulation of the invention may also be provided in the form of a kit, including instructions for use.

Administration of the therapeutic compositions and multipeptide formulations described above to an individual, preferably in non-immunogenic form, can be carried out using known procedures at dosages and for periods of time effective to cause down regulation of the immune response to Japanese cedar pollen or an allergen immunologically cross reactive with Japanese cedar pollen allergen (i.e., reduce the allergic symptoms caused by Japanese cedar pollen or related allergen including Japanese cedar pollen induced asthma) of the individual. Down regulation of the allergic immune response to Japanese cedar pollen in humans may be determined clinically whenever possible (see e.g., Varney et al, *British Medical Journal*, **302**:265-269 (1990), or may be determined subjectively

(i.e. the patient feels as if some or all of the allergic symptoms caused by Japanese cedar pollen have been alleviated).

One of the unique characteristics of each peptides of the invention is that each peptide possesses "superior solubility". Therefore, compositions and multipeptide formulations of the invention are particularly suitable for administration by injection (e.g. subcutaneous, or intravenous). However, optimized compositions and multipeptide formulations of the invention may be administered in any convenient manner wherein solubility of the active drug component is either desirable or acceptable, such as by injection (subcutaneous, intravenous, etc.), oral administration, sublingual, inhalation, transdermal application, rectal administration, or any other route of administration known in the art for administering soluble therapeutic agents. It may be desirable to administer simultaneously or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual as a single treatment episode. Each of such compositions for administration simultaneously or sequentially as a single treatment episode, may comprise only one unique peptide of the invention or may comprise an optimized multipeptide formulation in accordance with the invention.

For subcutaneous injection of one or more therapeutic compositions and multipeptide formulations of the invention, preferably about 1 μg- 3 mg and more preferably from about 20μg-1.5 mg, and even more preferably about 50 μg- 750 μg of each active component (peptide) per dosage unit may be administered. It is especially advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suited as unitary dosages for human subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the desired pharmaceutical carrier. The specification for the novel unit dosage forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of human subjects.

To administer a composition of the invention by other than parenteral administration, (i.e. by oral administration) it may be necessary to coat the composition with, or co-administer the composition with, a material to prevent its inactivation or enhance its absorption and bioavailability. For example, a peptide

formulation may be co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al., (1984) J. Neuroimmunol., 7:27). When a peptide is suitably protected, the peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The peptide and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, solutions, gels, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the composition and preparations may, of course, be varied and may conveniently be between about 5 to 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. In addition, the active compound may be incorporated into sustained-release or controlled release (steady state or pulsatile release) preparations and formulations.

Effective amounts of the optimized drug compositions of the invention will vary according to factors such as the degree of sensitivity of the individual to the antigen, the age, sex, and weight of the individual, and the ability of peptide to cause down regulation of the antigen specific immune response in the individual. Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered over the course of days, weeks, months or years, or the dose may be proportionally increased or reduced with each subsequent injection as indicated by the exigencies of the therapeutic situation. In one preferred therapeutic regimen, subcutaneous injections of therapeutic compositions are given once a week for 3-6 weeks. The dosage may remain constant for each injection or may increase or decrease with each subsequent injection. A booster injection may be administered at intervals of about three months to about one year after initial treatment and may involve only a single injection or may involve another series of injections similar to that of the initial treatment.

This invention is further illustrated by the following non-limiting examples.

Example 1
Japanese Cedar Pollen Allergic Patient T Cell Studies with overlapping Cry j I peptides.

Synthesis of Overlapping Peptides

Japanese cedar pollen *Cry j* I was divided into a set of 35 peptides of 20 amino acids in length and overlapping by 10 amino acids. Overlapping peptides were synthesized using standard Fmoc/tBoc synthetic chemistry and purified by Reverse Phase HPLC. Figure 2 shows the overlapping *Cry j* I peptides used in these studies. The peptide names are consistent throughout.

T Cell Responses to Cedar Pollen Antigenic Peptides

Peripheral blood mononuclear cells (PBMC) were purified by lymphocyte separation medium (LSM) centrifugation of 60 ml of heparinized blood from Japanese cedar pollen-allergic patients who exhibited clinical symptoms of seasonal rhinitis and were MAST and/or skin test positive for Japanese cedar pollen. Long term T cell lines were established by stimulation of 2 X 10^6 PBL/ml in bulk cultures of complete medium (RPMI-1640, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, $5x10^{-5}$M 2-mercaptoethanol, and 10 mM HEPES supplemented with 5% heat inactivated human AB serum) with 20 µg/ml of partially purified native *Cry j* I (75% purity containing three bands similar to the three bands in Fig. 2) for 7 days at 37°C in a humidified 5% CO_2 incubator to select for *Cry j* I reactive T cells. This amount of priming antigen was determined to be optimal for the activation of T cells from most cedar pollen allergic patients. Viable cells were purified by LSM centrifugation and cultured in complete medium supplemented with 5 units recombinant human IL-2/ml and 5 units recombinant human IL-4/ml for up to three weeks until the cells no longer responded to lymphokines and were considered "rested". The ability of the T cells to proliferate to selected peptides, recombinant *Cry j* I (r*Cry j* I), purified native *Cry j* I, or recombinant *Amb a* I.1 (r*Amb a*I.1) or a positive control, phyto-hemaglutinin (PHA) was then assessed. For assay, 2 X 10^4 rested cells were restimulated in the presence of 2 X 10^4 autologous Epstein-Barr virus (EBV)-transformed B cells (prepared as described below) (gamma-irradiated with 25,000 RADS) with 2-50 µg/ml of selected peptides, *Cry j* I, purified native *Cry j* I or r*Amb a* I.1 or PHA, in a volume of 200 µl complete medium in duplicate or triplicate wells in 96-well round bottom plates for 2-4 days. The optimal

incubation was found to be 3 days. Each well then received 1 μCi tritiated thymidine for 16-20 hours. The counts incorporated were collected onto glass fiber filter mats and processed for liquid scintillation counting. Data collected (not shown) indicated the effect of varying antigen dose in assays with recombinant *Cry j* I, purified native *Cry j* I, and recombinant *Amb a* I.1 and several antigenic peptides synthesized as described above. Some peptides were found to be inhibitory at high concentrations in these assays. The titrations were used to optimize the dose of peptides in T cell assays. The maximum response in a titration of each peptide is expressed as the stimulation index (S.I.). The S.I. is the counts per minute (CPM) incorporated by cells in response to peptide, divided by the CPM incorporated by cells in medium only. An S.I. value equal to or greater than 2 times the background level is considered "positive" and indicates that the peptide contains a T cell epitope. The positive results were used in calculating mean stimulation indices for each peptide for the group of patients tested. The results (data not shown) demonstrated that patient #999 responds well to recombinant *Cry j* I, and purified native *Cry j* I, as well as to peptides CJ1-2, 3, 20, and 22 but not to recombinant *Amb a* I.1. This indicates that *Cry j* I T cell epitopes are recognized by T cells from this particular allergic patient and that r*Cry j* I and peptides, 3 (SEQ ID NO: 49), 20 (SEQ ID NO: 50), and 22 (SEQ ID NO: 51) contain such T cell epitopes. Furthermore, the epitopes were often not detected with the adjacent overlapping peptides, and therefore probably span the non-overlapping central residues of the reactive peptides. No significant cross-reactivity was found in T cell assays using T cells primed with control antigens or with *Cry j* I primed T cells against other antigens.

The above procedure was followed with a number of other patients. Individual patient results were used in calculating the mean S.I. for each peptide if the patient responded to the *Cry j* I protein at an S.I. of 2.0 or greater and the patient responded to at least one peptide derived from *Cry j* I at an S.I. of 2.0 or greater. A summary of positive experiments from twenty-five patients is shown in Figure 4. The bars represent the positivity index. Above each bar is the percent of positive responses with an S.I. of at least two to the peptide or protein in the group of patients tested. In parenthesis above each bar are the mean stimulation indices for each peptide or protein for the group of patients tested. All twenty-five T cell lines responded to purified native *Cry j* I and 68.0% of the T cell lines responded to r*Cry j* I. These twenty-five T cell lines also responded at a significantly lower level to r*Amb a* I.1 indicating that the *Amb a* I allergens share a degree of

homology with *Cry j* I and that "shared" T cell epitopes might exist between *Cry j* I and *Amb a* I. This panel of Japanese cedar allergic patients responded to peptides CJ1-1 (SEQ ID NO: 1), CJ1-2 (SEQ ID NO: 2), CJ1-3 (SEQ ID NO: 3), CJ1-4 (SEQ ID NO: 4), CJ1-7 (SEQ ID NO: 7), CJ1-8 (SEQ ID NO: 8), CJ1-9 (SEQ ID NO: 9), CJ1-10 (SEQ ID NO: 10), CJ1-11 (SEQ ID NO: 11), CJ1-12 (SEQ ID NO: 12), CJ1-14 (SEQ ID NO: 14), CJ1-15 (SEQ ID NO: 15), CJ1-16 (SEQ ID NO: 16), CJ1-17 (SEQ ID NO: 17), CJ1-18 (SEQ ID NO: 18), CJ1-19 (SEQ ID NO: 19), CJ1-20 (SEQ ID NO: 20), CJ1-21 (SEQ ID NO: 21), CJ1-22 (SEQ ID NO: 22), CJ1-23 (SEQ ID NO: 23), CJ1-24 (SEQ ID NO: 24), CJ1-25 (SEQ ID NO: 25), CJ1-26 (SEQ ID NO: 26), CJ1-27 (SEQ ID NO: 27), CJ1-28 (SEQ ID NO: 28), CJ1-30 (SEQ ID NO: 30), CJ1-31 (SEQ ID NO: 31), CJ1-32 (SEQ ID NO: 32), CJ1-33 (SEQ ID NO: 33), CJ1-34 (SEQ ID NO: 34) and CJ1-35 (SEQ ID NO: 35) indicating that these peptides contain T cell epitopes.

Preparation of (EBV)-transformed B Cells for Use as Antigen Presenting Cells

Autologous EBV-transformed cell lines were γ-irradiated with 25,000 Rad and used as antigen presenting cells in secondary proliferation assays and secondary bulk stimulations. These EBV-transformed cell lines were made by incubating 5 X 10^6 PBL with 1 ml of B-59/8 Marmoset cell line (ATCC CRL1612, American Type Culture Collection, Rockville, MD) conditioned medium in the presence of 1 µg/ml phorbol 12-myristate 13-acetate (PMA) at 37°C for 60 minutes in 12 X 75 mm polypropylene round-bottom Falcon snap cap tubes (Becton Dickinson Labware, Lincoln Park, NJ). These cells were then diluted to 1.25 X 10^6 cells/ml in RPMI-1640 as described above except supplemented with 10% heat-inactivated fetal bovine serum and cultured in 200 µl aliquots in flat bottom culture plates until visible colonies were detected. They were then transferred to larger wells until the cell lines were established.

Example 2
Determination of percentage of total T cell reactivity and patient coverage of the combination of unique peptides of the invention.

Synthesis of unique peptides of the invention

Peptides CJI-24.5 (SEQ. ID. NO: 37) and CJI- 44.8 (SEQ. ID. NO: 38) were synthesized using standard Fmoc synthetic chemistry. Peptide CJI-43.39

(SEQ. ID. NO: 36) was synthesized by t-Boc synthetic chemistry. All peptides were purified to 93% or greater purity using the procedures for producing highly purified peptides desribed earlier. Figure 3 shows the unique *Cry j* I peptides used in these studies. The peptide names are consistent throughout.

T cell reactivity of the unique peptides of the invention:

Peptides CJI-24.5 (SEQ ID NO: 37), CJI-43.39 (SEQ ID NO: 36), and CJI-44.8 (SEQ ID NO: 38) were tested for T cell reactivity (data not shown) as described in Example 1 and were determined to elicit T cell activity as did each of their "parent" peptides from which they were derived and thus are suitable as candidate peptides for further study to determine if in combination, they possess a sufficient percentage of the total T cell reactivity of *Cry j* I in a sufficient percentage of the populations sensitive to *Cry j* I.

T cell studies with overlapping peptides and peptide candidates:

Secondary T cell cultures determined to be reactive with *Cry j* I protein antigen were derived from 36 *Cry j* I-allergic subjects and analyzed for reactivity to the overlapping set of peptides (Fig. 2) and the candidates peptides of the invention (Fig.3) in an in vitro T cell proliferation assay as described in Example 1. The results are shown in Fig. 5. The highest stimulation index greater than or equal to 2.0 in response to each peptide was recorded for each subject tested. The data were then analyzed by the equations described earlier in the specification.

The combination of candidate peptides CJI-24.5 (SEQ ID NO: 37), CJI-43.39 (SEQ ID NO: 36), and CJI-44.8 (SEQ ID NO: 38) had a range of T cell reactivity of abour 36%-53% based on an analysis of 36 patients (Fig. 5), the frequency of response at 97% represents reactivity to at least one of the candidate peptides, indicating that this combination of peptides fits the first criteria for "unique" peptides of the invention in that in combination peptides CJI-24.5 (SEQ ID NO: 37), CJI-43.39 (SEQ ID NO: 36), and CJI-44.8 (SEQ ID NO: 38), comprising a sufficient percentage of the total T cell reactivity of *Cry j* I in a substantial percentage of the population tested.

Example 3

IgE Binding Studies

To analyze IgE reactivity to candidate peptides CJI-24.5 (SEQ ID NO: 37), CJI-43.39 (SEQ ID NO: 36), and CJI-44.8 (SEQ ID NO: 38) and shown in Fig. 3,

a direct ELISA format was used. ELISA wells were coated with the candidate peptides and then assayed for IgE binding. The binding results were generated using two different pools of *Cry j* allergic patient plasma. Patient plasma pool A (denoted PHP-A) was formulated by mixing equal volumes of plasma from 22 patients that were all shown to be positive for direct IgE binding to native purified *Cry j* I by ELISA. The second pool (PHP-D) was formulated by the combination of equal plasma volumes from 8 patients that had IgE binding by direct ELISA to both native and denatured purified *Cry j* I. This pool was generated to increase the chance of detecting reactivity towards peptides. Both pools in this assay set showed direct binding to the native purified *Cry j* I (data not shown). There was no detectable IgE binding reactivity to any of the peptides tested at any of the plasma concentrations used. To control for the presence of peptide coating the wells, mouse polyclonal antisera was generated to the peptides. These antisera were then used in direct ELISA binding to demonstrate that the peptides were coating the wells. The results of these assays (data not shown) indicated that peptides were coating the wells.

Example 4

Simultaneous determination of pH-solubility and pH stability profiles of candidate peptides of the invention

1. Buffer Preparation

50 mM sodium phosphate stock solutions:

Stock solution A: 0.66 g (0.05 mol) of monobasic sodium phosphate monohydrate was dissolved in 100 mL of WFI. The solution was filtered through a 0.2 micron filter

Stock solution B: 0.71 g (0.05 mol) of dibasic sodium phosphate was disolved in 100ml WFI. The solution was filtered through a 0.2 micron filter.

2. Initial peptide dispersions

Dispersion A: 3.0 mg of each peptide CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38)and CJI-43.39 (SEQ. ID. NO: 36) was weighed out separately and placed in separate 1.5 mL eppendorf vials with 600 µL of stock solution A. The composition was agitated for 5 seconds to mix well.

Dispersion B: 3.0 mg of each peptide, CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38) and CJI-43.39 (SEQ. ID. NO: 36) was weighed out

separately and placed in separate 1.5 mL eppendoff vials with 600 µL of stock solution B. The mixture was agitated for 5 seconds to mix well.

Dispersions A and B were sonicated for 2 minutes for good homogeneity. A small volume was pipetted from each dispersion into a labeled eppendoff vial according to the following volume ratio:

Vial #	Suspension A (µL)	Suspension B (µL)	Total Volume (µL)	Estimated final pH
1	100	0	100	5.2
2	90	10	100	5.8
3	80	20	100	6.2
4	70	30	100	6.4
5	60	40	100	6.6
6	50	50	100	6.6
7	40	60	100	6.8
8	30	70	100	7.0
9	20	80	100	7.1
10	10	90	100	7.3
11	0	100	100	7.6

The resultant solutions/suspensions were stored in the dark at 22°C for 24 hours without agitation. 11 micro centrifuge filters were labeled on the retentate cup. The weight of each cup with the cap excluding the filter reservoir was measured. The equilibrated solution/suspension was pipetted from each eppendoff vial into the filer reservoir, and placed into micro centrifuge and spun for 10 minutes. After centrifugation, the filter reservoir was discarded and the weight of each cup/cap with the collected filtrate was recorded. The net weight of the filtrate in each cup was calculated by subtracting the weight of the empty cup/cap from the weight of the cup/cap containing the filtrate. The pH of the filtrates in each cup was measured using a micro combination pH electrode. After each pH reading, the electrode tip was rinsed with 1.0 mL of the selected dilution buffer into each cup. The total of diluted solution in each cup/cap was recorded. The dilution factors were calculated by dividing the weight of the diluted solution by the weight of the filtrate.

The concentration of peptide in the diluted solution was determined by HPLC analysis. The solubility of peptide at each pH was obtained by multiplying the measured concentration with the dilution factor. The extent of degradation of

peptides was estimated by calculating the percent of total degradant peak area over the total peak area.

The pH values with respect to the solubility values was plotted and are shown in Fig. 6a-c for each respective peptide. As shown in the solubility curve for each peptide as represented in Fig. 6a-c, peptides CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38) and CJI-43.39 (SEQ. ID. NO: 36) are each soluble at greater than 5 mg/ml at a pH in the pH range of pH-6 to pH 8.

The pH stability profiles for each peptide CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38) and CJI-43.39 (SEQ. ID. NO: 36) were calculated and tabulated as a function of total of degradant peak area (data not shown). None of the peptides showed any significant degradation after a 24 hour period.

Therefore, each of peptides CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38) and CJI-43.39 (SEQ. ID. NO: 36) was determined to possess the appropriate solubility and stability required of a unique peptide of the invention.

Although the invention has been described with reference to its preferred embodiments, other embodiments, can achieve the same results. Variations and modifications to the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modification and equivalents and follow in the true spirit and scope of this invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ImmuLogic Pharmaceutical Corporation

(ii) TITLE OF INVENTION: Pharmaceutical Formulations For Treating Japanese Cedar Pollen Allergy

(iii) NUMBER OF SEQUENCES: 51

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ImmuLogic Pharmaceutical Corporation, Inc.
(B) STREET: 610 Lincoln St
(C) CITY: Waltham
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02154

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: U.S. Serial No. 08/226,248
(B) FILING DATE: April 8, 1994

(A) APPLICATION NUMBER: U.S. Serial No. 08/350,225
(B) FILING DATE: December 6, 1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Darlene A. Vanstone
(B) REGISTRATION NUMBER: 35,729
(C) REFERENCE/DOCKET NUMBER: 025.7 PCT(IMI-028CPC)

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 466-6000
(B) TELEFAX: (617) 466-6040

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp Ser Asn Trp Ala Gln
1 5 10 15

Asn Arg Met Lys
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val
1 5 10 15

Gly Phe Gly Ser
20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Ala Asp Cys Ala Val Gly Phe Gly Ser Ser Thr Met Gly Gly Lys
1 5 10 15

Gly Gly Asp Leu
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val Thr Asn Ser
1 5 10 15

Asp Asp Asp Pro
20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Thr Val Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly
1 5 10 15

Thr Leu Arg Tyr
20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Asn Pro Ala Pro Gly Thr Leu Arg Tyr Gly Ala Thr Arg Asp Arg
1 5 10 15

Pro Leu Trp Ile
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn Met
1 5 10 15

Asn Ile Lys Leu
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ile Phe Ser Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile
1 5 10 15

Ala Gly Tyr Lys
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr Phe Asp Gly Arg Gly
1 5 10 15

Ala Gln Val Tyr
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro
1 5 10 15

Cys Val Phe Ile
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Gly Asn Gly Gly Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val
1 5 10 15

Ile Ile His Gly
20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Lys Arg Val Ser Asn Val Ile Ile His Gly Leu Tyr Leu Tyr Gly Cys
1 5 10 15

Ser Thr Ser Val
20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Tyr Leu Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile
1 5 10 15

Asn Glu Ser Phe
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Leu Gly Asn Val Leu Ile Asn Glu Ser Phe Gly Val Glu Pro Val His
1 5 10 15

Pro Gln Asp Gly
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu Arg
1 5 10 15

Thr Ala Thr Asn
20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ala Leu Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn
1 5 10 15

Ser Phe Ser Asn
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser Ser Asp Gly Leu Val
1 5 10 15

Asp Val Thr Leu
20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val Thr
1 5 10 15

Ile Ser Asn Asn
20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Thr Ser Thr Gly Val Thr Ile Ser Asn Asn Leu Phe Phe Asn His His
1 5 10 15

Lys Val Met Leu
20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His Asp Asp Ala
1 5 10 15

Tyr Ser Asp Asp
20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Leu Gly His Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr
1 5 10 15

Val Ala Phe Asn
20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Lys Ser Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn Cys
1 5 10 15

Gly Gln Arg Met
20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr Gly
1 5 10 15

Leu Val His Val
20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Pro Arg Ala Arg Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp
1 5 10 15

Pro Trp Thr Ile
20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr Ala Ile Gly Gly Ser
1 5 10 15

Ser Asn Pro Thr
20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Tyr Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn
1 5 10 15

Ser Phe Thr Ala
20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ile Leu Ser Glu Gly Asn Ser Phe Thr Ala Pro Asn Glu Ser Tyr Lys
1 5 10 15

Lys Gln Val Thr
20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile Gly Cys Lys
1 5 10 15

Thr Ser Ser Ser
20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ile Arg Ile Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp
1 5 10 15

Gln Ser Thr Gln
20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Ser Asn Trp Val Trp Gln Ser Thr Gln Asp Val Phe Tyr Asn Gly
1 5 10 15

Ala Tyr Phe Val
20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu
1 5 10 15

Gly Gly Asn Ile
20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala
1 5 10 15

Phe Asn Val Glu
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn Gly Asn Ala Thr Pro
1 5 10 15

Gln Leu Thr Lys
20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr
1 5 10 15

Cys Ser Leu Ser
20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asn Ala Gly Val Leu Thr Cys Ser Leu Ser Lys Arg Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe
1 5 10 15

Asn Gln Phe Gly Asp Glu
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Asp Lys Glu Pro Arg Ala Arg Tyr Gly Leu Val His Val Ala Asn Asn
1 5 10 15

Asn Tyr Asp Pro Trp Thr Ile Glu Glu Glu
20 25

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Asp Glu Glu Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly
Gly
1 5 10 15

Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu
20 25

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1337 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Crytpomeria japonica

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 66..1187

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 129..1187

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGTCAATCTG CTCATAATCA TAGCATAGCC GTATAGAAAG AAATTCTACA CTCTGCTACC
60

AAAAA ATG GAT TCC CCT TGC TTA GTA GCA TTA CTG GTT TTC TCT TTT
107
Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe
-21 -20 -15 -10

GTA ATT GGA TCT TGC TTT TCT GAT AAT CCC ATA GAC AGC TGC TGG AGA
155

```
Val Ile Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg
-5                          1                   5

GGA GAC TCA AAC TGG GCC CAA AAT AGA ATG AAG CTC GCA GAT TGT GCA
203
Gly Asp Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala
 10                  15                  20                  25

GTG GGC TTC GGA AGC TCC ACC ATG GGA GGC AAG GGA GGA GAT CTT TAT
251
Val Gly Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr
                    30                  35                  40

ACG GTC ACG AAC TCA GAT GAC GAC CCT GTG AAT CCT GCA CCA GGA ACT
299
Thr Val Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr
                45                  50                  55

CTG CGC TAT GGA GCA ACC CGA GAT AGG CCC CTG TGG ATA ATT TTC AGT
347
Leu Arg Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser
            60                  65                  70

GGG AAT ATG AAT ATA AAG CTC AAA ATG CCT ATG TAC ATT GCT GGG TAT
395
Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr
        75                  80                  85

AAG ACT TTT GAT GGC AGG GGA GCA CAA GTT TAT ATT GGC AAT GGC GGT
443
Lys Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly
 90                  95                  100                 105

CCC TGT GTG TTT ATC AAG AGA GTT AGC AAT GTT ATC ATA CAC GGT TTG
491
Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu
                110                 115                 120

TAT CTG TAC GGC TGT AGT ACT AGT GTT TTG GGG AAT GTT TTG ATA AAC
539
Tyr Leu Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn
            125                 130                 135

GAG AGT TTT GGG GTG GAG CCT GTT CAT CCT CAG GAT GGC GAT GCT CTT
587
Glu Ser Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu
        140                 145                 150

ACT CTG CGC ACT GCT ACA AAT ATT TGG ATT GAT CAT AAT TCT TTC TCC
635
Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser
    155                 160                 165

AAT TCT TCT GAT GGT CTG GTC GAT GTC ACT CTT ACT TCG ACT GGA GTT
683
Asn Ser Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val
170                 175                 180                 185

ACT ATT TCA AAC AAT CTT TTT TTC AAC CAT CAT AAA GTG ATG TTG TTA
731
Thr Ile Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu
                190                 195                 200

GGG CAT GAT GAT GCA TAT AGT GAT GAC AAA TCC ATG AAG GTG ACA GTG
779
Gly His Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val
            205                 210                 215

GCG TTC AAT CAA TTT GGA CCT AAC TGT GGA CAA AGA ATG CCC AGG GCA
827
Ala Phe Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala
        220                 225                 230
```

```
CGA TAT GGA CTT GTA CAT GTT GCA AAC AAT AAT TAT GAC CCA TGG ACT
875
Arg Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr
    235                 240                 245

ATA TAT GCA ATT GGT GGG AGT TCA AAT CCA ACC ATT CTA AGT GAA GGG
923
Ile Tyr Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly
250                 255                 260                 265

AAT AGT TTC ACT GCA CCA AAT GAG AGC TAC AAG AAG CAA GTA ACC ATA
971
Asn Ser Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile
                270                 275                 280

CGT ATT GGA TGC AAA ACA TCA TCA TCT TGT TCA AAT TGG GTG TGG CAA
1019
Arg Ile Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln
            285                 290                 295

TCT ACA CAA GAT GTT TTT TAT AAT GGA GCT TAT TTT GTA TCA TCA GGG
1067
Ser Thr Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly
        300                 305                 310

AAA TAT GAA GGG GGT AAT ATA TAC ACA AAG AAA GAA GCT TTC AAT GTT
1115
Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val
    315                 320                 325

GAG AAT GGG AAT GCA ACT CCT CAA TTG ACA AAA AAT GCT GGG GTT TTA
1163
Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu
330                 335                 340                 345

ACA  TGC  TCT  CTC  TCT  AAA  CGT  TGT  TGATGATGCA  TATATTCTAG
CATGTTGTAC     1217
Thr Cys Ser Leu Ser Lys Arg Cys
                350

TATCTAAATT    AACATCAACA    AGAAAATATA    TCATGATGTA    TATTGTTGTA
TTGATGTCAA     1277

AATAAAAATG    TATCTTTTAC    TATTAAAAAA    AAAAATGATC    GATCGGACGG
TACCTCTAGA     1337
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe Val Ile
-21 -20                 -15                 -10

Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly Asp
 -5                   1                   5                  10

Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala Val Gly
                15                  20                  25

Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr Thr Val
            30                  35                  40
```

```
Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr Leu Arg
 45                  50                  55

Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn
 60                  65                  70                  75

Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr
                 80                  85                  90

Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys
             95                 100                 105

Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu Tyr Leu
        110                 115                 120

Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn Glu Ser
    125                 130                 135

Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu Thr Leu
140                 145                 150                 155

Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser Asn Ser
                160                 165                 170

Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val Thr Ile
            175                 180                 185

Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His
        190                 195                 200

Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val Ala Phe
    205                 210                 215

Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala Arg Tyr
220                 225                 230                 235

Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile Tyr
                240                 245                 250

Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly Asn Ser
            255                 260                 265

Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile Arg Ile
        270                 275                 280

Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln Ser Thr
    285                 290                 295

Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr
300                 305                 310                 315

Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu Asn
                320                 325                 330

Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr Cys
            335                 340                 345

Ser Leu Ser Lys Arg Cys
        350
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys Thr Phe Asp Gly Arg Gly
1 5 10 15

Ala Gln Val Tyr Ile Gly Asn Gly Gly Pro Cys Val Phe Ile
20 25 30

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Asp Glu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser
1 5 10 15

Asn Ser Ser Asp Asp
20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu
1 5 10 15

Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu
20 25 30

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile
1 5 10 15

Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu

20 25

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile
1 5 10 15

Tyr Thr Lys Lys Glu Ala Phe Asn
20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Asp Glu Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly Gly
1 5 10 15

Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Ala Glu
20 25

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Asp Glu Glu Asn Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly
1 5 10 15

Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu
20 25

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Asp Glu Gly Ala Tyr Phe Val Ser Ser Gly Lys Tyr Glu Gly Gly Asn
1 5 10 15

Ile Tyr Thr Lys Lys Glu Ala Phe Asn Ala Glu
20 25

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Leu Ala Asp Cys Ala Val Gly Phe Gly Ser Ser Thr Met Gly Gly Lys
1 5 10 15

Gly Gly Asp Leu
20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Leu Phe Phe Asn His His Lys Val Met Leu Leu Gly His Asp Asp Ala
1 5 10 15

Tyr Ser Asp Asp
20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Lys Ser Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn Cys
1 5 10 15

Gly Gln Arg Met
20

What is claimed is:

1. An isolated peptide of *Cry j* I having an amino acid sequence selected from the group consisting of: CJI-43.39 (SEQ. ID. NO: 36), CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38).

2. A therapeutic composition comprising at least one isolated *Cry j* I peptide selected from the group consisting of: CJI-43.39 (SEQ. ID. NO: 36), CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38).

3. A method of treating sensitivity to Japanese Cedar Pollen allergen or an allergen immunologically cross reactive with Japanese cedar pollen allergen comprising administering sequentially or simultaneously at least two different compositions of claim 2.

4. A method of treating sensitivity to Japanese cedar pollen allergen or an allergen immunologically cross-reactive with Japanese cedar pollen allergen comprising administering sequentially or simultaneously peptides: CJI-43.39 (SEQ. ID. NO: 36), CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38) in a pharmaceutically acceptable form.

5. A multipeptide formulation for pharmaceutical administration comprising at least two peptides of *Cry j* I selected from the group consisting of: CJI-43.39 (SEQ. ID. NO: 36), CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38); each peptide having T cell activity, each peptide being soluble and stable at a physiologically acceptable predetermined pH; and a pharmaceutically acceptable excipient.

6. The multipeptide formulation of claim 5 further comprising a pharmaceutically acceptable counter ion.

7. The multipeptide formulation of claim 5 wherein said predetermined pH is in the range of about pH 5.5 to pH 7.5.

8. The multipeptide formulation of claim 5 comprising peptides CJI-42.5 (SEQ. ID. NO: 42), CJI-43.39 (SEQ. ID. NO: 36), CJI-24.5 (SEQ. ID. NO: 37), CJI-44.8 (SEQ. ID. NO: 38).

9. The composition of claim 2 wherein said T cell activity is at least 39%.

10. An optimized multipeptide formulation suitable for therapeutic treatment of humans suffering from allergy to Japanese Cedar Pollen comprising:

Cry j I peptides CJ1-24.5 (SEQ. ID. NO: 37), CJ1-43.39 (SEQ. ID. NO: 36) and CJ1-44.8 (SEQ. ID. NO: 38), each peptide having a concentration of 0.75 mg per peptide;

0.05 M Sodium Phosphate pH 6.0-8.0;

5% w/v Mannitol, U.S.P.; and

Sterile Water for Injection, U.S.P.

11. The optimized multipeptide formulation of claim 10 wherein said formulation is in the form of a lyophilized powder.

12. The optimized multipeptide formulation of claim 10 wherein the final pH of the formulation is in the pH range of pH 7.0 to pH 7.5.

13. Use of a therapeutic composition of claim 2 for the manufacture of a medicament for treatment of sensitivity of an individual to Japanese cedar pollen allergen, or an allergen immunologically cross reactive with Japanese cedar pollen allergen.

14. Use of the multipeptide formulation of claim 5 for the manufacture of a medicament for treatment of sensitivity of an individual to Japanese cedar pollen allergen, or an allergen immunologically cross reactive with Japanese cedar pollen allergen.

15. Use of the optimized multipeptide formulation of claim 10 for the manufacture of a medicament for treatment of sensitivity of an individual to Japanese cedar pollen allergen, or an allergen immunologically cross reactive with Japanese cedar pollen allergen

```
5'-AGTCAATCTG CTCATAATCA TAGCATAGCC GTATAGAAAG AAATTCTACA CTCTGCTACC    60

AAAAA ATG GAT TCC CCT TGC TTA GTA GCA TTA CTG GTT TTC TCT TTT          107
      Met Asp Ser Pro Cys Leu Val Ala Leu Leu Val Phe Ser Phe
      -21 -20                 -15                 -10

GTA ATT GGA TCT TGC TTT TCT GAT AAT CCC ATA GAC AGC TGC TGG AGA        155
Val Ile Gly Ser Cys Phe Ser Asp Asn Pro Ile Asp Ser Cys Trp Arg
         -5                  1               5

GGA GAC TCA AAC TGG GCC CAA AAT AGA ATG AAG CTC GCA GAT TGT GCA        203
Gly Asp Ser Asn Trp Ala Gln Asn Arg Met Lys Leu Ala Asp Cys Ala
 10                  15                  20                  25

GTG GGC TTC GGA AGC TCC ACC ATG GGA GGC AAG GGA GGA GAT CTT TAT        251
Val Gly Phe Gly Ser Ser Thr Met Gly Gly Lys Gly Gly Asp Leu Tyr
                 30                  35                  40

ACG GTC ACG AAC TCA GAT GAC GAC CCT GTG AAT CCT GCA CCA GGA ACT        299
Thr Val Thr Asn Ser Asp Asp Asp Pro Val Asn Pro Ala Pro Gly Thr
             45                  50                  55

CTG CGC TAT GGA GCA ACC CGA GAT AGG CCC CTG TGG ATA ATT TTC AGT        347
Leu Arg Tyr Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser
         60                  65                  70
```

Fig. 1a

```
GGG AAT ATG AAT ATA AAG CTC AAA ATG CCT ATG TAC ATT GCT GGG TAT      395
Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr
     75                  80                  85

AAG ACT TTT GAT GGC AGG GGA GCA CAA GTT TAT ATT GGC AAT GGC GGT      443
Lys Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly
 90                  95                 100                 105

CCC TGT GTG TTT ATC AAG AGA GTT AGC AAT GTT ATC ATA CAC GGT TTG      491
Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Leu
                110                 115                 120

TAT CTG TAC GGC TGT AGT ACT AGT GTT TTG GGG AAT GTT TTG ATA AAC      539
Tyr Leu Tyr Gly Cys Ser Thr Ser Val Leu Gly Asn Val Leu Ile Asn
            125                 130                 135

GAG AGT TTT GGG GTG GAG CCT GTT CAT CCT CAG GAT GGC GAT GCT CTT      587
Glu Ser Phe Gly Val Glu Pro Val His Pro Gln Asp Gly Asp Ala Leu
        140                 145                 150

ACT CTG CGC ACT GCT ACA AAT ATT TGG ATT GAT CAT AAT TCT TTC TCC      635
Thr Leu Arg Thr Ala Thr Asn Ile Trp Ile Asp His Asn Ser Phe Ser
    155                 160                 165
```

Fig. 1b

```
AAT TCT TCT GAT GGT CTG GTC GAT GTC ACT CTT ACT TCG ACT GGA GTT          683
Asn Ser Ser Asp Gly Leu Val Asp Val Thr Leu Thr Ser Thr Gly Val
170                 175                 180                 185

ACT ATT TCA AAC AAT CTT TTT TTC AAC CAT CAT AAA GTG ATG TTG TTA          731
Thr Ile Ser Asn Asn Leu Phe Phe Asn His His Lys Val Met Leu Leu
                190                 195                 200

GGG CAT GAT GAT GCA TAT AGT GAT GAC AAA TCC ATG AAG GTG ACA GTG          779
Gly His Asp Asp Ala Tyr Ser Asp Asp Lys Ser Met Lys Val Thr Val
            205                 210                 215

GCG TTC AAT CAA TTT GGA CCT AAC TGT GGA CAA AGA ATG CCC AGG GCA          827
Ala Phe Asn Gln Phe Gly Pro Asn Cys Gly Gln Arg Met Pro Arg Ala
        220                 225                 230

CGA TAT GGA CTT GTA CAT GTT GCA AAC AAT AAT TAT GAC CCA TGG ACT          875
Arg Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Trp Thr
    235                 240                 245

ATA TAT GCA ATT GGT GGG AGT TCA AAT CCA ACC ATT CTA AGT GAA GGG          923
Ile Tyr Ala Ile Gly Gly Ser Ser Asn Pro Thr Ile Leu Ser Glu Gly
250                 255                 260                 265
```

Fig. 1c

```
AAT AGT TTC ACT GCA CCA AAT GAG AGC TAC AAG AAG CAA GTA ACC ATA          971
Asn Ser Phe Thr Ala Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr Ile
                270                 275                 280

CGT ATT GGA TGC AAA ACA TCA TCA TCT TGT TCA AAT TGG GTG TGG CAA         1019
Arg Ile Gly Cys Lys Thr Ser Ser Ser Cys Ser Asn Trp Val Trp Gln
            285                 290                 295

TCT ACA CAA GAT GTT TTT TAT AAT GGA GCT TAT TTT GTA TCA TCA GGG         1067
Ser Thr Gln Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val Ser Ser Gly
        300                 305                 310

AAA TAT GAA GGG GGT AAT ATA TAC ACA AAG AAA GAA GCT TTC AAT GTT         1115
Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val
    315 320 325

GAG AAT GGG AAT GCA ACT CCT CAA TTG ACA AAA AAT GCT GGG GTT TTA         1163
Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu
330                 335                 340                 345

ACA TGC TCT CTC TCT AAA CGT TGT TGATGATGCA TATATTCTAG CATGTTGTAC        1217
Thr Cys Ser Leu Ser Lys Arg Cys
                350

TATCTAAATT AACATCAACA AGAAAATATA TCATGATGTA TATTGTTGTA TTGATGTCAA       1277

AATAAAAATG TATCTTTTAC TATTAAAAAA AAAAATGATC GATCGGACGG TACCTCTAGA-3'    1337
```

Fig. 1d

PEPTIDE NAME	
CJI-1 (1-20)	DNPIDSCWRGDSNWAQNRMK
CJI-2 (11-30)	DSNWAQNRMKLADCAVGFGS
CJI-3 (21-40)	LADCAVGFGSSTMGGKGGDL
CJI-4 (31-50)	STMGGKGGDLYTVTNSDDDP
CJI-5 (41-60)	YTVTNSDDDPVNPAPGTLRY
CJI-6 (51-70)	VNPAPGTLRYGATRDRPLWI
CJI-7 (61-80)	GATRDRPLWIIFSGNMNIKL
CJI-8 (71-90)	IFSGNMNIKLKMPMYIAGYK
CJI-9 (81-100)	KMPMYIAGYKTFDGRGAQVY
CJI-10 (91-110)	TFDGRGAQVYIGNGGPCVFI
CJI-11 (101-120)	IGNGGPCVFIKRVSNVIIHG
CJI-12 (111-130)	KRVSNVIIHGLYLYGCSTSV
CJI-13 (121-140)	LYLYGCSTSVLGNVLINESF
CJI-14 (131-150)	LGNVLINESFGVEPVHPQDG
CJI-15 (141-160)	GVEPVHPQDGDALTLRTATN
CJI-16 (151-170)	DALTLRTATNIWIDHNSFSN
CJI-17 (161-180)	IWIDHNSFSNSSDGLVDVTL
CJI-18 (171-190)	SSDGLVDVTLTSTGVTISNN
CJI-19 (181-200)	TSTGVTISNNLFFNHHKVML
CJI-20 (191-210)	LFFNHHKVMLLGHDDAYSDD
CJI-21 (201-220)	LGHDDAYSDDKSMKVTVAFN
CJI-22 (211-230)	KSMKVTVAFNQFGPNCGQRM
CJI-23 (221-240)	QFGPNCGQRMPRARYGLVHV
CJI-24 (231-250)	PRARYGLVHVANNNYDPWTI
CJI-25 (241-260)	ANNNYDPWTIYAIGGSSNPT
CJI-26 (251-270)	YAIGGSSNPTILSEGNSFTA
CJI-27 (261-280)	ILSEGNSFTAPNESYKKQVT
CJI-28 (271-290)	PNESYKKQVTIRIGCKTSSS
CJI-29 (281-300)	IRIGCKTSSSCSNWVWQSTQ
CJI-30 (291-310)	CSNWVWQSTQDVFYNGAYFV
CJI-31 (301-320)	DVFYNGAYFVSSGKYEGGNI
CJI-32 (311-330)	SSGKYEGGNIYTKKEAFNVE
CJI-33 (321-340)	YTKKEAFNVENGNATPQLTK
CJI-34 (331-350)	NGNATPQLTKNAGVLTCSLS
CJI-35 (341-353)	NAGVLTCSLSKRC

Fig. 2

RECTIFIED SHEET (RULE 91)
ISA/EP

PEPTIDE NAME:	AMINO ACID SEQUENCE
CJI-43.39	DDAYSDDKSMKVTVAFNQFGDE
CJr-24.5	DKEPRARYGLVHVANNNYDPWTIEEE
CJI-44.8	DEEGAYFVSSGKYEGGNIYTKKEAFNVE

Fig. 3

RECTIFIED SHEET (RULE 91)
ISA /EP

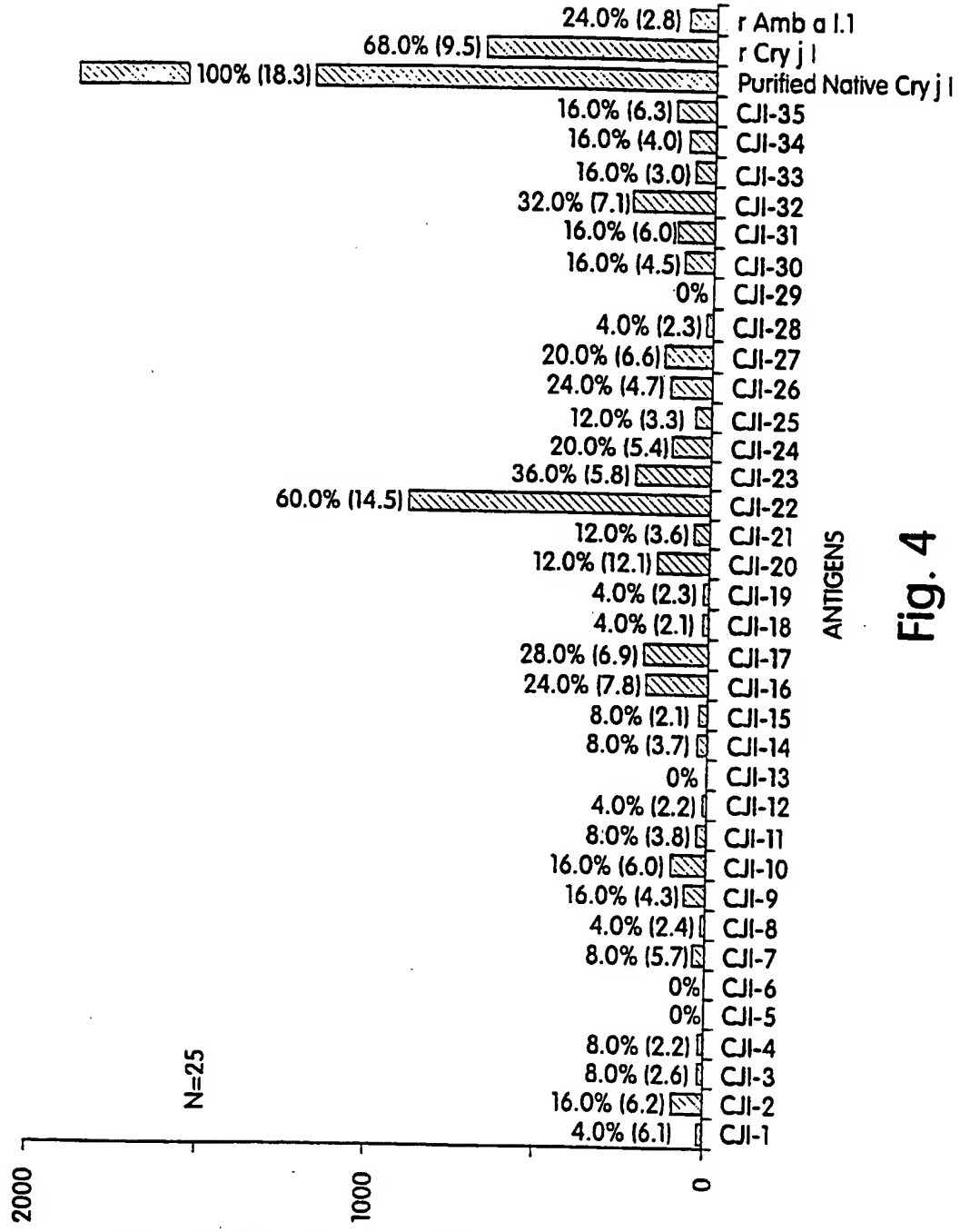

Fig. 4

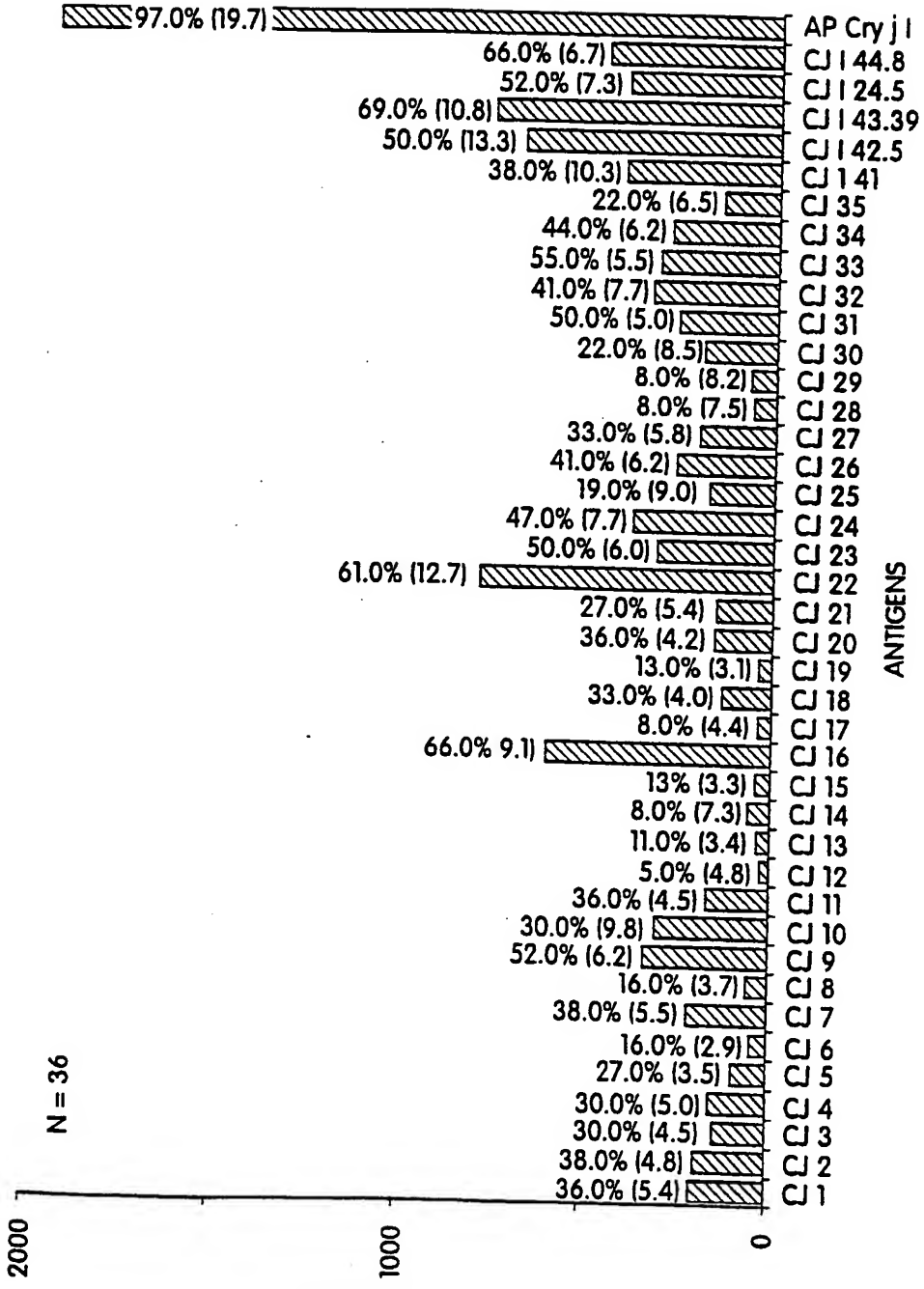

Fig. 5

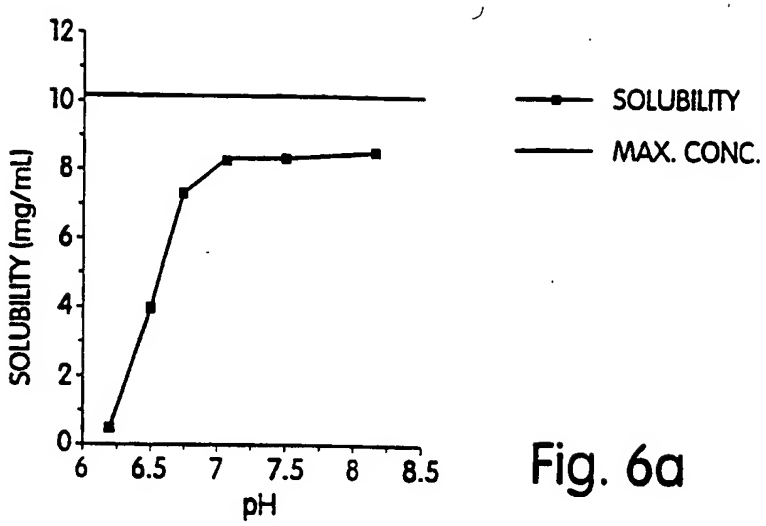

Fig. 6a

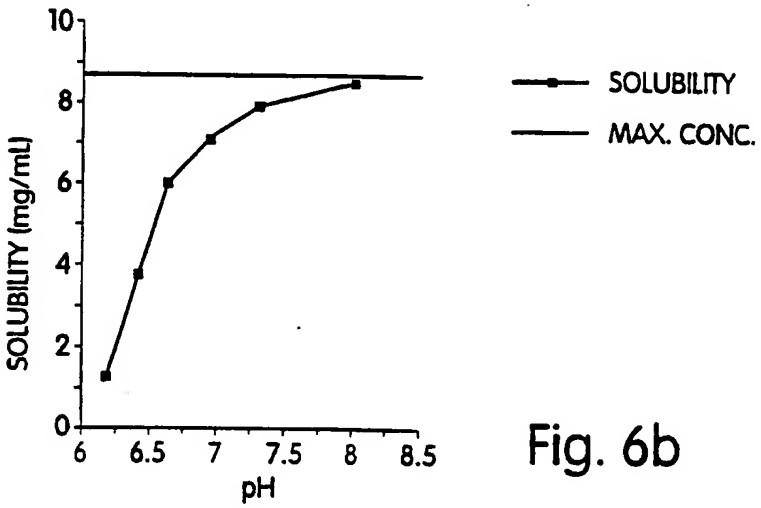

Fig. 6b

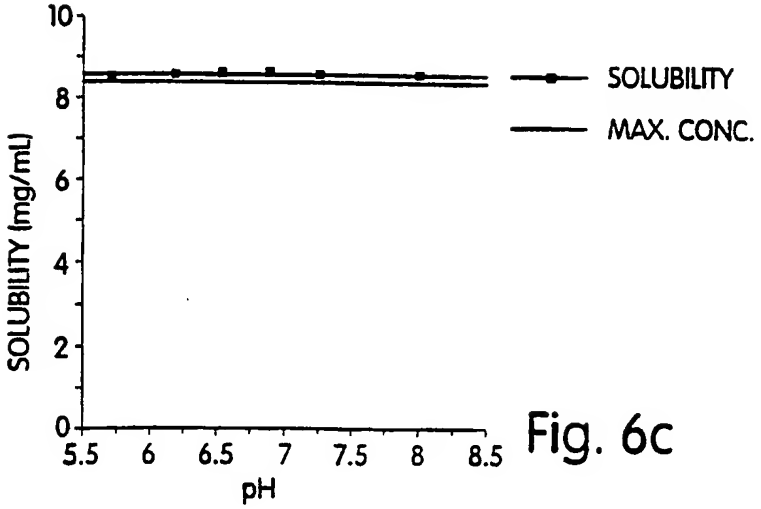

Fig. 6c

RECTIFIED SHEET (RULE 91)
ISA/EP

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/04249

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C07K14/415 A61K39/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO-A-94 01560 (IMMULOGIC PHARMACEUTICAL CORPORATION) 20 January 1994 cited in the application see figure 18 ---	1-15
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 199, no. 2, 15 March 1994 ORLANDO, FL US, pages 619-625, T.SONE ET AL. 'Cloning and sequencing of a cDNA clone coding for Cry j I, a major allergen of Japanese cedar pollen' see the whole document -----	1,2,5-15

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 1995	10.08.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04249

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 3,4
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 3 and 4 are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 95/04249

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9401560	20-01-94	AU-B-	3438493	31-01-94
		EP-A-	0595855	11-05-94
		JP-T-	6508994	13-10-94
		AU-A-	2300492	11-02-93
		CA-A-	2112913	21-01-93
		WO-A-	9301213	21-01-93
		CA-A-	2120954	20-01-94
		EP-A-	0659214	28-06-95